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Relationship Between IL-10 Gene Polymorphism and Spinal Tuberculosis

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Background: To investigate the relation between interleukin-10 (IL-10) gene rs1800871 (A/G) polymorphism and spinal tuberculosis.

Material/Methods: A total of 129 patients with spinal tuberculosis (spinal tuberculosis group) and 106 healthy subjects receiving physical examination (control group) were enrolled in this study. The general data of these subjects were collected, and the C-reactive protein, erythrocyte sedimentation rate (ESR) and baseline hematologic function were examined. The rs1800871 (A/G) polymorphism in IL-10 gene was detected by TaqMan-MGB probe method.

Results: The C-reactive protein, ESR, white blood cell count, absolute neutrophil count and relative neutrophil count in spinal tuberculosis group were higher than those in control group, while the absolute lymphocyte count and relative lymphocyte count were lower than those in control group ($p<0.05$). Compared with AA genotype, GG and AG+GG genotypes showed statistically significant difference in distribution frequency ($p<0.05$), but no significant difference was detected between AG genotype and AA genotype ($p>0.05$). In spinal tuberculosis group, the frequency of G allele was higher than that of A allele ($p<0.01$). The C-reactive protein, ESR, white blood cell count and relative neutrophil count in GG genotype were increased compared with those in AG+GG genotype ($p<0.05$).

Conclusions: The rs1800871 (A/G) polymorphism in IL-10 gene is related to the susceptibility to spinal tuberculosis. Moreover, carrying G allele increases the risk of spinal tuberculosis.

MeSH Keywords: Polymorphism, Single Nucleotide • Receptors, Interleukin-10 • Tuberculosis, Spinal

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Background

Tuberculosis is a chronic infectious disease resulting from the infection with mycobacterium tuberculosis. The secondary infection of bones and joints accounts for 10–35% of extrapulmonary tuberculosis, and spinal tuberculosis accounts for 50%, making it the most representative extrapulmonary tuberculosis [1]. However, spinal tuberculosis has become the leading cause of spinal spasm and deformity due to the overlong conservative treatment and surgical difficulty in spinal tuberculosis [2]. Epidemiological surveys have indicated that about 1/3 of people around the world are infected with tubercle bacilli, but only a few of them are attacked [3]. It may be related to the living environment and living habits of patients as well as the genetic susceptibility of the disease. Studies on the genetic susceptibility of patients to the disease help identify high-risk groups at the early stage and are conducive to early diagnosis and intervention, reducing the incidence rate of the disease. Studies have found that when mycobacterium tuberculosis infects the host and controls the inflammation progress, inflammation-related cytokines play important roles. As a cytokine in the acute phase of inflammation, interleukin-10 (IL-10) is involved in the down-regulation of inflammatory responses by interlacing with other relevant inflammatory factors, thereby affecting the development of spinal tuberculosis [4]. Currently, the association between spinal tuberculosis patients and IL-10 gene polymorphisms is not studied. Therefore, in this study, patients with spinal tuberculosis in our department were enrolled, and rs1800871 polymorphism in IL-10 gene was detected using TaqMan-minor groove binder (MGB) probe method, so as to explore the correlation between IL-10 gene polymorphism and spinal tuberculosis, providing theoretical support for genetic polymorphism of spinal tuberculosis.

Material and Methods

Objects of study

Spinal tuberculosis patients receiving treatment in our hospital from June 2016 to June 2018 were selected. Inclusion criteria: Patients 1) with typical symptoms of mycobacterium tuberculosis infection, such as magersucht, weakness and fever, 2) with positive result in tuberculin skin test, and diagnosed with spinal tuberculosis based on medicine imaging and pathological examinations, and 3) with complete clinical data and willing to cooperate in this study. Exclusion criteria: Patients 1) with dysfunction of important organs like heart, kidney or liver, 2) with immune diseases, or 3) with malignant tumors. According to the above criteria, 129 patients with spinal tuberculosis were enrolled in this study, including 66 males and 63 females with a mean age of (36.32±10.50) years old. Meanwhile, 106 healthy people in physical examination center in the corresponding period were selected as controls, including 50 males and 56 females with an average age of (40.80±6.54) years old. The study was approved by the hospital ethics committee (11/6/2016). All objects of study were unrelated Chinese Han population and signed the informed consent.

Study methods

Collection of general clinical data

The following data of subjects were collected: name, age, gender, C-reactive protein, erythrocyte sedimentation rate (ESR) and baseline hematologic function (white blood cell count, absolute neutrophil count, relative neutrophil count, absolute lymphocyte count, relative lymphocyte count, absolute monocyte count and relative monocyte count).

Extraction of deoxyribonucleic acid (DNA)

Elbow venous blood (1 mL) was collected from patients, and DNA was extracted with a medium-dose whole blood genomic DNA extraction kit (Beijing Bioteke Corporation, lot number: 0020170714) according to the instructions of the kit. Then, an ultra-micro ultraviolet spectrophotometer (Nanodrop-2000) was employed to measure the purity and concentration of DNA. The purity and concentration of all DNA samples met experimental requirements. Next, genotyping assays were performed on samples using a TaqMan® single nucleotide polymorphism (SNP) Genotyping Assays kit (Thermo, lot number: 1712101) (Specific gene locus probe information is shown in Table 1).

Statistical methods

Statistical Product and Service Solutions (SPSS) 20.0 software was used for statistical analyses. Measurement data were expressed as ($\bar{\chi}\pm s$), and independent-sample *t*-test was employed for comparisons between two groups. Likelihood ratio χ^2 test was applied to determine whether the distribution of each genotype was consistent with the Hardy-Weinberg equilibrium law. R×C table χ^2 test was adopted to compare the frequency of every genotype and allele. $p<0.05$ suggested that the difference was statistically significant.

Results

Comparisons of basic data

There were no statistically significant differences in age and gender between two groups ($p>0.05$), and they were comparable (Table 2).

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Table 1. TaqMan®-MGB probe information at locus rs1800871 of in IL-10 gene.

SNP reference	rs1800871
Assay ID	C___1747362_10
Protein ID	NP_000563.1
Single nucleotide polymorphism (SNP) Type	Intron
Context sequence	AGTGAGCAAACTGAGGCACAGAGAT[A/G] TTACATCACCTGTACAAGGGTACAC

Table 2. Comparisons of basic data between the two groups ($\overline{\chi}\pm s$).

Group	n	Age (years old)	Male/Female
Spinal tuberculosis group	129	36.32±10.50	66/63
Control group	106	40.80±6.54	50/56
t/χ^2		1.592	0.371
p		0.213	0.542

Table 3. Comparisons of blood examination indicators between the two groups ($\overline{\chi}\pm s$).

Index	Spinal tuberculosis group	Control group	t	p
C-reactive protein (mg/L)	24.5±1.5	3.0±1.1	5.21	0.001
ESR (mm/h)	54.4±11.3	3.59±2.1	7.58	0.000
White blood cell count ($\times10^9$)	8±2.317	6±2.324	2.459	0.032
Absolute neutrophil count ($\times10^9$)	6±1.609	4±1.594	1.164	0.049
Relative neutrophil count (%)	67.11±7.04	56.86±5.04	2.428	0.031
Absolute lymphocyte count ($\times10^9$)	1±4.187	2±5.649	2.578	0.029
Relative lymphocyte count (%)	22.57±7.49	33.29±5.87	2.743	0.018
Absolute monocyte count ($\times10^9$)	5±2.365	5±1.879	0.137	0.876
Relative monocyte count (%)	8.17±2.36	7.65±1.79	0.536	0.612

Table 4. Genetic equilibrium test of rs1800871genotypes in IL-10 gene.

Group	n	AA		AG		GG		χ^2	p
		Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency	Actual frequency	Theoretical frequency		
Spinal tuberculosis group	129	30	28.84	62	64.31	37	35.84	0.17	0.92
Control group	106	40	37.44	46	51.11	20	17.44	1.06	0.59

Comparisons of blood examination indicators

Compared with those in control group, the C-reactive protein, ESR, white blood cell count, absolute neutrophil count and relative neutrophil count in spinal tuberculosis group were increased, while the absolute lymphocyte count and relative lymphocyte count were decreased, showing statistically significant differences ($p<0.05$) (Table 3).

Genetic equilibrium test

The actual and theoretical frequencies of three genotypes in both spinal tuberculosis group and control group were subjected to likelihood ratio χ^2 test. The genotype frequencies of rs1800871 in IL-10 gene in spinal tuberculosis group and control group were consistent with the Hardy-Weinberg genetic equilibrium law ($p>0.05$), which were comparable (Table 4).

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Table 5. Comparisons of rs1800871genotypes in IL-10 gene [n (%)].

Genotype	Spinal tuberculosis group	Control group	OR (95% CI)	p
AA	30 (23.26)	40 (37.74)		
AG	62 (48.06)	46 (43.40)	0.556 (0.303–1.022)	0.058
GG	37 (28.68)	20 (18.86)	0.405 (0.197–0.834)	0.013
AG+GG	99 (76.74)	66 (62.26)	0.500 (0.284–0.881)	0.016

Table 6. Comparison of allele distribution in IL-10 gene rs1800871A/G between the two groups [n (%)].

Group	n	Allele [n (%)]		χ^2	p
		A	G		
Spinal tuberculosis group	129	122 (47.29)	136 (52.71)	6.890	0.009
Control group	106	126 (59.43)	86 (40.57)		

Table 7. Correlation analyses of rs1800871A/G genotypes in IL-10 gene and quantitative data in spinal tuberculosis group.

Item	GG	AG+GG	χ^2	p
C-reactive protein (mg/L)	28.5±1.7	26.4±1.2	2.447	0.011
ESR (mm/h)	64.4±10.7	60.6±12.3	1.823	0.042
White blood cell count ($\times 10^9$)	9±2.816	9±1.517	2.067	0.048
Absolute neutrophil count ($\times 10^9$)	6±1.816	6±1.709	1.354	0.178
Relative neutrophil count (%)	69.16±7.89	67.17±6.14	1.878	0.041
Absolute lymphocyte count ($\times 10^9$)	1±4.276	1±4.383	0.046	0.927
Relative lymphocyte count (%)	22.32±7.75	22.48±6.97	1.345	0.176

Comparison of distribution frequency of genotypes

The distribution frequencies of AA, AG and GG genotypes were 23.26%, 48.06% and 28.68% in spinal tuberculosis group, and 37.74%, 43.40% and 18.86% in control group, respectively. The distribution frequency of GG and AG+GG genotypes was different from that of AA genotype, and the difference was statistically significant ($p<0.05$). There was no significant difference between AG genotype and AA genotype based on comparison ($p>0.05$) (Table 5).

Comparison of distribution frequency of alleles

The distribution frequencies of A and G alleles in spinal tuberculosis group were 47.29% and 52.71%, respectively. In control group, the distribution frequencies of A and G alleles were 59.43% and 40.57%, respectively. Moreover, the distribution frequency of G allele was higher than that of A allele in spinal tuberculosis group ($p<0.01$) (Table 6).

Analyses on the correlations of genotypes with biochemical indicators

The relationships of GG and AG+GG genotypes with biochemical indicators were further analyzed based on the results of comparisons of biochemical indicators and genotypes between spinal tuberculosis group and control group. The C-reactive protein, ESR, white blood cell count and relative neutrophil count of GG genotype were higher than those of AG+GG genotype ($p<0.05$). However, no differences were found in the absolute neutrophils count, absolute lymphocyte count and relative lymphocyte count between two genotypes ($p>0.05$) (Table 7).

Discussion

Spinal tuberculosis is a chronic secondary tissue injury disease mainly caused by the entry of mycobacterium tuberculosis into the spine via the blood circulation after pulmonary tuberculosis or lymphatic tuberculosis, dominated by vertebral

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tuberculosis. Besides, it frequently occurs in adults and may cause spinal cord injury or even paraplegia in sever cases [5]. In this study, the comparisons of blood examination indicators between spinal tuberculosis group and control group revealed that spinal tuberculosis group had increased C-reactive protein, ESR, white blood cell count, absolute neutrophil count and relative neutrophil count, but reduced absolute lymphocyte count and relative lymphocyte count in comparison with control group, implying that patients with spinal tuberculosis have relatively low immunity and are more susceptible to infection, thus leading to increases in inflammation indexes. People susceptible to tuberculosis should pay more attention to improving immunity.

Currently, the correlations of spinal tuberculosis with susceptibility genes are studied, and it is reported that the incidence rate of tuberculosis in identical twins is clearly higher than that in fraternal twins [6]. In addition, genetic studies on Chinese patients with spinal tuberculosis conducted by Chinese scholars have revealed that the polymorphism of genes including mannose-binding lectin 2 (MBL2), cluster of differentiation 14 (CD14), tumor necrosis factor-alpha (TNF-α), human leucocyte antigen-DQA1 (HLA-DQA1) and IL-12 are interrelated with the susceptibility to spinal tuberculosis [7-10]. This shows that spinal tuberculosis is associated with genetic factors.

IL-10, as an important anti-inflammatory and immunoregulatory cytokine found on mouse helper T2 (Th2) cells by Mosmannand et al. [11], is able to inhibit the production of interferon-gamma (IFN-γ) cytokines by suppressing Th1 cells. Moreover, IL-10 gene is located on chromosome 1 q31-32 and contains 5 exons and 4 introns, with a molecular weight of 5.3 kb. IL-10 mainly exerts its biological functions through the janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway. Firstly, IL-10 binds to the R1 receptor and exposes the binding site of R1 to IL-10 R2. Then, it binds to R2 to form a complex receptor. The interaction of R1 and R2 activates intracellular JAK1 and tyrosine kinase 2 (TyK2), triggering the phosphorylation of tyrosine residues Y446 and Y496 on JAK1. Activated Y446 and Y496 bind to the transcription factors STAT1, STAT3 and STAT5 to activate transcription factors, and the activated transcription factors enter into cells to regulate the expressions of genes [12,13]. In addition to the JAK-STAT pathway, IL-10 gene can inhibit nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) to affect inhibitor of NF-κB (IκB) kinase activity and DNA binding activity [14,15], thus adjusting gene expression and ultimately regulating the proliferation and differentiation of cells. Recent studies have manifested that IL-10 is closely related to the onset of autoimmune diseases such as systemic lupus erythematosus, type I diabetes, primary immune thrombocytopenia and inflammatory bowel disease [16–19]. The research results of Xue Haibin [20] showed that dysregulated IL-10 expression influences the allergic reaction of spinal tuberculosis, and IL-10 is involved in the fibrosis of spinal tuberculosis and osteogenesis, controlling the spread of tuberculosis lesions and prolonging the time of bone regeneration. It is thus clear that IL-10 is also associated with the onset of spinal tuberculosis.

In this study, rs1800871 (A/G) polymorphism in IL-10 was selected, and the frequencies of genotypes and alleles in spinal tuberculosis group and control group were analyzed using the TaqMan-MGB probe method. The results showed that GG genotype, AG+GG genotype and G allele were related to the pathogenesis of spinal tuberculosis, suggesting that the recessive mode and cumulative mode are suitable for describing the genetic model of IL-10 rs1800871 in spinal tuberculosis. GG recessive homozygous mutations in IL-10 gene rs1800871 (A/G) result in increased onset risk of spinal tuberculosis, and carrying G allele significantly enhances the risk of spinal tuberculosis.

Conclusions

This study further analyzed the correlations of GG and AG+GG genotypes with biochemical indicators in spinal tuberculosis group, and it was found that the C-reactive protein, ESR, white blood cell count and relative neutrophil count of GG genotype were higher than those of AG+GG genotype, while the absolute neutrophil count, absolute lymphocyte count and relative lymphocyte count of them were similar, indicating that GG recessive homozygous mutations possibly affect the expressions of inflammatory factors through different pathways in spinal tuberculosis, having evident regulatory effects on the expressions of inflammation indexes (C-reactive protein, ESR, white blood cell count and neutrophil) and relatively weak regulatory impact on lymphocyte.

Conflict interest

None.

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References:

1. Allie N, Grivennikov SI, Keeton R et al: Prominent role for T cell-derived tumour necrosis factor for sustained control of *Mycobacterium tuberculosis* infection. Sci Rep, 2013, 3(5): 1809
2. Gulland A: More cases of tuberculosis than previously thought, WHO reports. BMJ, 2016; 355: i5562
3. Dye C, Williams BG: The population dynamics and control of tuberculosis. Science, 2010; 328(5980): 856–61
4. Liu C, Zhan X, Xiao Z et al: Transcript levels of major interleukins in relation to the clinicopathological profile of patients with tuberculous intervertebral discs and healthy controls. PLoS One, 2014; 9(6): e101324
5. Francisco NM, Hsu NJ, Keeton R et al: TNF-dependent regulation and activation of innate immune cells are essential for host protection against cerebral tuberculosis. J Neuroinflammation, 2015; 12(1): 125
6. van der Eijk EA, van de Vosse E, Vandenbroucke JP, van Dissel JT: Heredity versus environment in tuberculosis in twins: The 1950s United Kingdom Prophit Survey Simonds and Comstock revisited. Am J Respir Crit Care Med, 2007; 176(12): 1281–88
7. Zheng M, Shi S, Wei W et al: Correlation between MBL2/CD14/TNF-α gene polymorphisms and susceptibility to spinal tuberculosis in Chinese population. Biosci Rep, 2018; 38(1): BSR20171140
8. Shen J, Shi S, Lai Z: Identification of HLA-DQA1 as a susceptibility gene for spinal tuberculosis by exome sequencing. Med Sci Monit, 2018; 24: 3442–49
9. Roth DE, Soto G, Arenas F et al: Association between vitamin D receptor gene polymorphisms and response to treatment of pulmonary tuberculosis. J Infect Dis, 2004; 190(5): 920–27
10. Tso HW, Lau YL, Tam CM et al: Associations between IL12B polymorphisms and tuberculosis in the Hong Kong Chinese population. J Infect Dis, 2004; 190(5): 913–19
11. Ramakrishnan V, Akram Husain RS, Ahmed SS: Genetic predisposition of IL-10 promoter polymorphisms with risk of multiple sclerosis: A meta-analysis. J Neuroimmunol, 2017; 306: 11–18
12. Khare V, Paul G, Movadat O et al: IL10R2 Overexpression promotes IL22/STAT3 signaling in colorectal carcinogenesis. Cancer Immunol Res, 2015; 3(11): 1227–35
13. Song X, Wang CT, Geng XH: MicroRNA-29a promotes apoptosis of monocytes by targeting STAT3 during sepsis. Genet Mol Res, 2015; 14(4): 13746–53
14. Song S, Bi J, Wang D et al: Porcine reproductive and respiratory syndrome virus infection activates IL-10 production through NF-κB and p38 MAPK pathways in porcine alveolar macrophages. Dev Comp Immunol, 2013; 39(3): 265–72
15. Hovsepian E, Penas F, Siffo S et al: IL-10 inhibits the NF-κB and ERK/MAPK-mediated production of pro-inflammatory mediators by up-regulation of SOCS-3 in trypanosoma cruzi-infected cardiomyocytes. PLoS One, 2013; 8(11): e79445
16. Yuan C, Chen WX, Zhu JS et al: IL-10 treatment is associated with prohibitin expression in the Crohn's disease intestinal fibrosis mouse model. Mediators Inflamm, 2013; 2013: 617145
17. Osada Y, Fujiyama T, Kamimura N et al: Dual genetic absence of STAT6 and IL-10 does not abrogate anti-hyperglycemic effects of *Schistosoma mansoni*, in streptozotocin-treated diabetic mice. Exp Parasitol, 2017; 177: 1–12
18. Heinemann K, Wilde B, Hoerning A et al: Decreased IL-10(+) regulatory B cells (Bregs) in lupus nephritis patients. Scand J Rheumatol, 2016; 45(4): 312–16
19. Hua F, Ying L, Xin Z et al: The expression profile of toll-like receptor signaling molecules in CD19 +, B cells from patients with primary immune thrombocytopenia. Immunol Lett, 2016; 176: 28–35
20. Haibin X: TNF-alpha, IFN-gamma, TGF-beta and IL-10 expression in the spinal tuberculosis and its effect on the degree of disease: Institute of military medical treatment of the Chinese people's Liberation Army (PLA), 2009

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